Their Use

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# FIGURE 1A

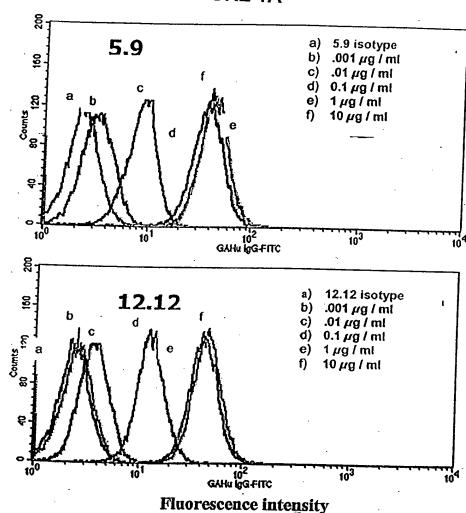
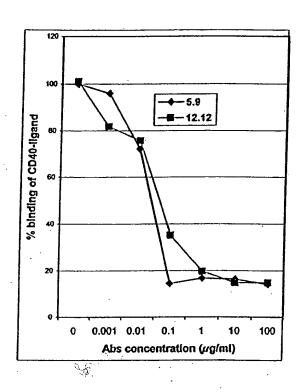


FIGURE 1B

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**FIGURE 2A** 

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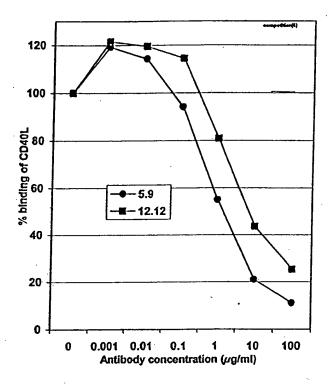


FIGURE 2B

Their Use

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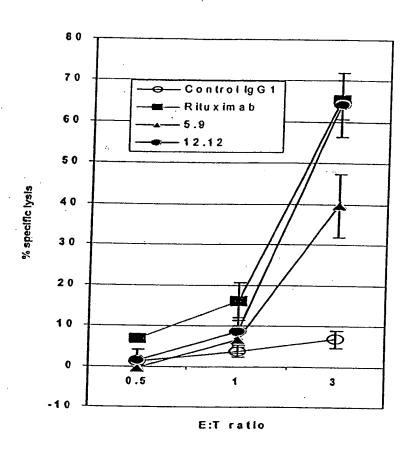


FIGURE 3A

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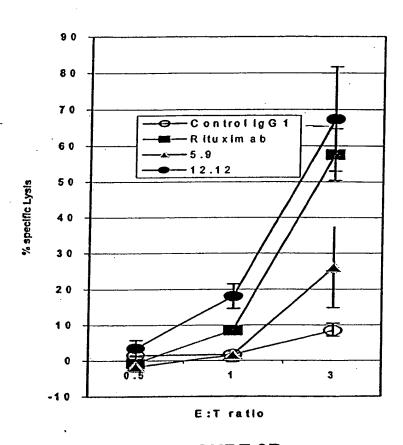


FIGURE 3B



10

Days after tumor inoculation

Tumor volume (Mean

mm3±SEM)

1500

1000

500-

0

Dosing

5

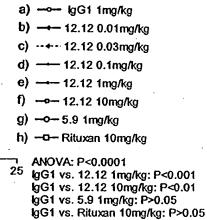


FIGURE 4

20

15

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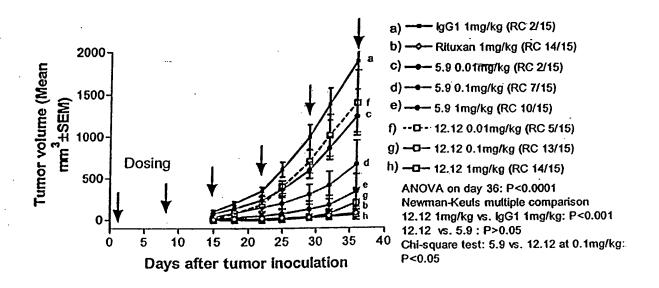
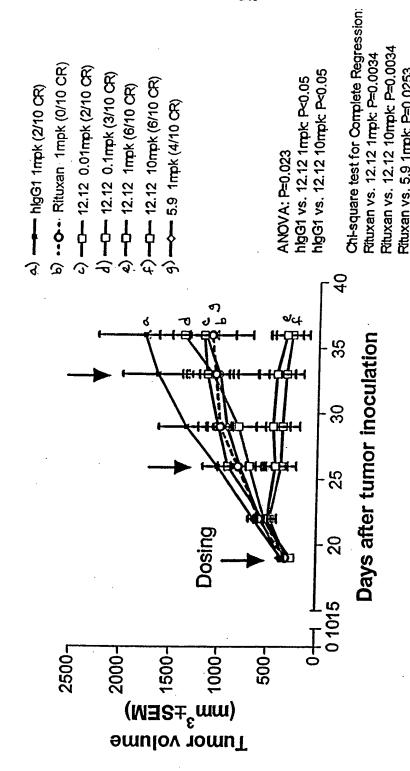


FIGURE 5

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# Number of CD20 and CD40 Molecules on Namalwa and Daudii Cells

Methods:
1. Harvest and wash cells once with PBS w/o Ca++//Mg++ plus 0.5%BSA and 0.1% Sodium Azide.
2. Block 185 cells with 10% huserum in PBS w/o Ca++/Mg++ plus 0.1% Sodium Azide on ice for 30 minutes.
3. Stain cells with FITC conjugated antibodies (12.12-FTC or Rituximab-FTC) on ice for 40minutes. Cells were also stained with huloG1-FTC
for non-specific binding control. Antibody concentrations were 0.01, 0.1, 1, 10 and 100ug per ml.
4. Determine Mean Channel Fluorescence (Geometric Mean) by flow cytometer using log amplifier. PI was added to exclude dead cells.
5. Determine Mean Channel Fluorescence (Geometric Means) of Quantum M24FITC (3,000 to 5,000 MESF*)
Quantum TM <sup>25</sup> FITC (50,000 to 2,000,000 MESF) and Quantum TM26FITC (10,000 to 500,000 MESF)
at the same instrument settings as for samples analysis.
MESF: Molecules of Equivalent Soluble Fluorochrome
6. Construct calibration curve by plotting MESF (y-axis) vs. the Geometric Means (x-axis).
7. The number of molecules per cell was determined using the following equation: y=ax/b where v is equal to MESF and
x is equal to Mean Channel Fluorescence of the sample. Mean Channel Fluorescence used for each sample was
the Geo Mean at saturation concentration (12.12FTIC) or the highest concentration (rituximabFTTC).
the antibody binding capacity (ABC). ABC of hulgGFITC of respected sample was corrected to obtain the
linal antibody binding capacatly.
The state of the s

·	Daudii		Namalwa	
Exp.	CD40	CD20	CD40	CD20
E090403	14403.0	93676.5	3296.4	6200.1
E091003	13214.9	108438.5	3081.5	4788.2
E091103	13702.6	100509.1	3165.7	3988.3
E091203	13278.9	128158.3	3164.9	4618.0
Average	13,649.9	107,695.6	3,177.1	4.898.7
Stdev	546.7	14915.9	88.8	933.4

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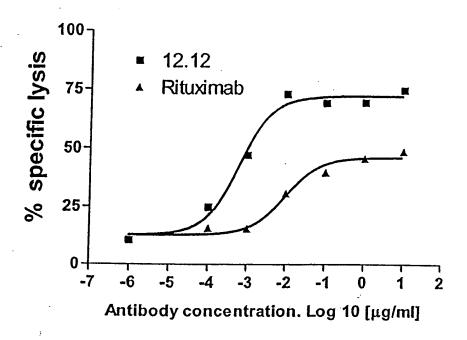


FIGURE 8

Their Use

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### FIGURE 9A

### CHIR 12.12 light chain:

leader:

MALPAQLLGLLMLWVSGSSG

variable:

DIVMTQSPLSLTVTPGEPASISCRSSQSLLYSNGYNYLDWYLQKPGQSPQVLISLGSNRASG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQARQTPFTFGPGTKVDIR

constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

### FIGURE 9B

### CHIR-12.12 heavy chain:

leader:

MEFGLSWVFLVAILRGVQC

variable:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYEESNRYHAD SVKGRFTISRDNSKITLYLQMNSLRTEDTAVYYCARDGGIAAPGPDYWGQGTLVTVSS

constant:

ASTKGPSVFPLAPASKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTOKSLSLSPGK

alternative constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK

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### FIGURE 10A

DNA sequence of light chain of CHIR-12.12:

### FIGURE 10B

DNA sequence of heavy chain of CHIR-12.12 (including introns):

ggtccagcctgggaggtccctgagactctcctgtgcagcctctggattcaccttcagtagctatggcatgcactgggtccgccaggctccaggcaaggggctggagtgggtggcagttatatcatatgaggaaagtaatagataccatgcagactccgtgaagggccgattcacca teteeagagacaatteeaagateaegetgtatetgeaaatgaaeageeteagaaetgaggaeaeggetgtgtattaetgtgegagagat gggggtatagcagcacctgggcctgactactggggccagggaaccctggtcaccgtctcctcagcaagtaccaagggcccatccgt cttcccctggcgcccgctagcaagagcacctctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccgg cagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtgg gctatgcagtccagtccaggcaggcaggaggcccgtctgcctcttcacccggaggcctctgcccactcatgctcagg getgggetcagacetgecaagagecatatecgggaggaceetgeccetgacetaagcecaaceceaaaggecaaactetecactecet cage to ggacac et to te ce caga atte caga tace cea at et te te te geaga ge cea a at et t g t gacaa a acte a caca t geaga general descriptions and the term of the term ofaggccccagccgggtgctgacacgtccacctccatctcttcctcagcacctgaactcctggggggaccgtcagtcttcctcttccccccaaaacccaaggacacctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtca ccatcg agaaaaccatctccaa agccaa aggtgggacccgtggggtgcgagggccacatggacagaggccggctcggcccaccctetgecetgagagtgacegetgtaceaacetetgtecetacagggcageceegagaaceacaggtgtacaceetgececcatecegg gaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaa tgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctatagcaagctcaccgtggac aagagcaggtggcagcaggggaacgtetteteatgeteegtgatgeatgaggetetgeacaaccaetacaegcagaagagcetetee ctgtctccgggtaaatga3'

Title: Antagonist Anti-CD40 Monoclonal Antibodies and Methods for Their Use Inventor(s): Long et al.

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### FIGURE 11A

CHIR-5.9 light chain:

leader:

MALLAQLLGLLMLWVPGSSG

variable:

AIVMTQPPLSSPVTLGQPASISCRSSQSLVHSDGNTYLNWLQQRPGQPPRLLIYKFFRRLSG VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQVTQFPHTFGQGTRLEIK

constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

### FIGURE 11B

CHIR-5.9 heavy chain:

leader:

MGSTAILALLLAVLQGVCA

variable:

EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSP SFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARGTAAGRDYYYYYGMDVWGQGTTVTVS S

constant:

ASTKGPSVFPLAPASKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK

alternative constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK

Their Use

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### FIGURE 12A

Coding sequence for short isoform of human CD40:

- 1 atggttegte tgeetetgea gtgegteete tggggetget tgetgaeege tgteeateea
- 61 gaaccaccca etgeatgeag agaaaaacag tacetaataa acagteagtg etgttetttg
- 121 tgccagccag gacagaaact ggtgagtgac tgcacagagt tcactgaaac ggaatgcctt
- 181 cettgeggtg aaagegaatt eetagacace tggaacagag agacacactg ceaceageac
- 241 aaatactgeg acceeaacet agggettegg gteeageaga agggeacete agaaacagae
- 301 accatetgea cetgtgaaga aggetggeae tgtaegagtg aggeetgtga gagetgtgte
- 361 etgeaceget eatgetegee eggetttggg gteaageaga ttgetaeagg ggtttetgat
- 421 accatetgeg agecetgeee agteggette ttetecaatg tgteatetge tttegaaaaa
- 481 tgtcaccett ggacaaggte eecaggateg getgagagee etggtggtga tecceateat
- 541 cttcgggatc ctgtttgcca tcctcttggt gctggtcttt atcaaaaagg tggccaagaa
- 601 gccaaccaat aa

### FIGURE 12B

Encoded short isoform of human CD40:

- 1 mvrlplqcvl wgclltavhp epptacrekq ylinsqccsl cqpgqklvsd cteftetecl
- 61 pcgesefldt wnrethchqh kycdpnlglr vqqkgtsetd tictceegwh ctseacescv
- 121 lhrscspgfg vkqiatgvsd ticepcpvgf fsnvssafek chpwtrspgs aespggdphh
- 181 lrdpvchplg aglyqkggqe anq

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### FIGURE 12C

### Coding sequence for long isoform of human CD40:

- 1 atggttcgtc tgcctctgca gtgcgtcctc tggggctgct tgctgaccgc tgtccatcca
- 61 gaaccaccca etgeatgeag agaaaaacag tacetaataa acagteagtg etgttetttg
- 121 tgccagccag gacagaaact ggtgagtgac tgcacagagt tcactgaaac ggaatgcctt
- 181 cettgeggtg aaagegaatt cetagacace tggaacagag agacacactg ceaccagcac
- 241 aaatactgcg accccaacct agggettcgg gtccagcaga agggeacete agaaacagac
- 301 accatetgea cetgtgaaga aggetggeae tgtacgagtg aggeetgtga gagetgtgte
- 361 ctgcaccgct catgctcgcc cggctttggg gtcaagcaga ttgctacagg ggtttctgat
- 421 accatetgeg agecetgece agteggette ttetecaatg tgteatetge tttegaaaaa
- 481 tgtcaccett ggacaagetg tgagaccaaa gacetggttg tgcaacagge aggcacaaac
- 541 aagactgatg ttgtctgtgg teeccaggat eggetgagag eeetggtggt gateeceate
- 601 atctteggga teetgtttge eateetettg gtgetggtet ttateaaaaa ggtggeeaag
- 661 aagccaacca ataaggcccc ccaccccaag caggaacccc aggagatcaa ttttcccgac
- 721 gatetteetg geteeaacae tgetgeteea gtgeaggaga etttacatgg atgeeaaceg
- 781 gtcacccagg aggatggcaa agagagtcgc atctcagtgc aggagagaca gtga

### FIGURE 12D

### Encoded long isoform of human CD40:

- 1 myrlplqcvl wgclltavhp epptacrekq ylinsqccsl cqpgqklvsd cteftetecl
- 61 pcgesefldt wnrethchqh kycdpnlglr vqqkgtsetd tictceegwh ctseacescv
- 121 lhrscspgfg vkqiatgvsd ticepcpvgf fsnvssafek chpwtscetk dlvvqqagtn
- 181 ktdvvcgpqd rlralvvipi ifgilfaill vlvfikkvak kptnkaphpk qepqeinfpd
- 241 dlpgsntaap vqetlhgcqp vtqedgkesr isvqerq

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## FIGURE 13

